EFFICACY OF ANTIHYPOXIC DRUGS REDUCING THE AFFINITY OF HEMOGLOBIN FOR OXYGEN IN ACUTE CEREBRAL ISCHEMIA

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The end result of measures aimed at alleviating circulatory disorders in cerebral ischemia is an improvement of the oxygen supply to the brain tissue [2]. One way of preventing and treating cerebral ischemia is the use of agents leading to weakening of the affinity of hemoglobin (Hb) for oxygen and increasing the supply of oxygen to the tissues [10]. The promising nature of this approach to the prevention and treatment of cerebral ischemia is due both to the "economy" of the method (a shift of p_{50} by 1 mm Hg is equivalent to a change of 10% in the cerebral blood flow [15]), and to the significant reduction in the efficacy of vasodilators during ischemia [9]. However, the rationale of the use of drugs with this kind of mechanism of action in circulatory cerebral hypoxia has not been investigated; it is not yet clear to what degree a preparation must be able to reduce the affinity of Hb for O_2 in order to exhibit its antihypoxic activity in acute cerebral ischemia.

The aim of this investigation was to determine the effect of various antihypoxic agents on the affinity of Hb for O_2 and the possible role of this phenomenon in the alleviation of hypoxia of brain tissue in acute cerebral ischemia.

EXPERIMENTAL METHOD

Experiments were carried out on 74 noninbred rats weighing 250-300 g under pentobarbital anesthesia (1 g/kg, intraperitoneally) in two series. In the animals of Series I, arterial blood samples were taken by catheterization of the carotid artery. Blood samples were obtained before and 1 h after intraperitoneal injection of the preparations: sodium hydroxybutyrate (100 mg/kg), ascorbate (100 mg/kg), cavinton (ethyl ester of apovincamic acid) (5 mg/kg), bemitil (50 mg/kg), and ethomersol (50 mg/kg). To evaluate the effect of the drugs on binding of Hb with O_2, we used a method proposed by ourselves, which consisted of estimating the value of HbO_2 in control and experimental blood samples after saturation with a gas mixture containing 4% O_2, 5% CO_2, and 91% N_2 [6]. Values of pO_2, pCO_2, pH, and Hb and HbO_2 concentrations in the blood samples were monitored by means of an ABL-4 gas analyzer. P_{50} was calculated by the formula in [14]. In the experiments of Series II both carotid arteries of the rats were dissected and ligatures applied to them. The animals were then turned over, the head was fixed in a halter, and scalped. To record pO_2 a platinum electrode made from wire 0.2 mm in diameter, insulated throughout its length except 2 mm at the tip, was implanted into the cerebral cortex through a burr-hole in the parietal bone. The dynamics of pO_2 was recorded on a ZP-9 polarograph. Acute cerebral ischemia (hypoxia) was induced by tying the ligatures on the two carotid arteries simultaneously. The drugs, in the above-mentioned doses, were injected intraperitoneally 30 min before ligation. The results were subjected to statistical analysis by Student's t-test and Wilcoxon's nonparametric test [3].

EXPERIMENTAL RESULTS

Choice of the drugs used in the investigation was based on the following considerations. All compounds, in the doses used, possess antihypoxic activity; in the case of most of them, this was revealed under the conditions of circulatory cerebral

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TABLE 1. Effect of Intraperitoneal Injection of Sodium Hydroxybutyrate, Ascorbate, Cavinton, Bemitil, and Ethomersol on pH, pCO_2, pO_2, HbO_2, and p_{50} in Blood of Rats After Saturation with Gas Mixture Containing 4% O_2, 5% CO_2, and 91% N_2 (M ± m)

Preparation	Time of determination	pO_2, mm Hg	pCO_2, mm Hg	pH	HbO_2, %	P_{50}, mm Hg
Sodium hydroxybutyrate (n = 6)	Before injection	30,4±0,6	36,7±0,3	7,338±0,022	49,0±0,7	28,9±0,5
	After injection	30,2±0,5	37,0±0,4	7,349±0,028	47,9±0,9	29,6±0,4
Ascorbate (n = 5)	Before injection	29,8±0,4	38,0±0,5	7,350±0,019	49,7±0,6	28,4±0,4
	After injection	29,9±0,4	37,8±0,4	7,312±0,027	46,6±0,8*	29,5±0,4*
Cavinton (n = 6)	Before injection	30,7±0,4	37,5±0,5	7,347±0,021	50,0±0,9	28,9±0,5
	After injection	31,1±0,6	36,8±0,4	7,338±0,019	45,5±0,6*	31,1±0,4*
Bemitil (n = 5)	Before injection	29,2±0,5	36,5±0,5	7,339±0,020	50,2±0,9	27,1±0,6
	After injection	29,2±0,4	37,2±0,4	7,310±0,036	45,2±0,8*	29,4±0,5*
Ethomersol (n = 6)	Before injection	29,4±0,4	37,0±0,4	7,339±0,015	49,2±1,0	28,0±0,5
	After injection	29,5±0,5	37,2±0,3	7,281±0,028	40,9±1,1*	30,7±0,5*
Physiological saline (n = 5)	Before injection	30,1±0,4	36,8±0,4	7,340±0,024	49,2±0,7	28,7±0,4
	After injection	29,9±0,5	37,1±0,4	7,332±0,031	49,0±0,6	28,3±0,4

Legend. *p < 0.05 (calculated by Student's t test).

TABLE 2. Effect of Sodium Hydroxybutyrate, Ascorbate, Cavinton, Bemitil, and Ethomersol on pO_2 in Brain Tissue After Circulatory Hypoxia for 30 min

Preparation	Background	30 min of circulatory hypoxia
	%	
Sodium hydroxybutyrate (n = 6)	100	39
Ascorbate (n = 6)	100	38
Cavinton (n = 9)	100	42*
Bemitil (n = 6)	100	46*
Ethomersol (n = 7)	100	75*
Control (n = 7)	100	35

Legend. Mean values are given. *p < 0.05 compared with control (calculated by Wilcoxon's nonparametric test).

hypoxia [4, 5, 8, 11]. In the case of ascorbate, cavinton, and bemitil, the ability of these substances to facilitate the release of oxygen by hemoglobin has been described [2, 7, 13].

For some of the drugs tested, significant differences in the effect on affinity of Hb for O_2 were found (Table 1). After saturation of the blood with the gas mixture, a very small decrease in the HbO_2 level compared with the control was observed only with sodium hydroxybutyrate (by 1.8%; $p > 0.05$). Under the influence of ascorbate, cavinton, bemitil, and ethomersol, HbO_2 fell by 3.0%, 4.5g %, 5.0%, and 8.3% respectively ($p < 0.05$).

Under the conditions of this model of acute cerebral ischemia (toward 30 min of occlusion of both carotid arteries in the rats) the test drugs also weakened hypoxia in the cerebral cortex to different degrees (Table 2). Sodium hydroxybutyrate and ascorbate did not exhibit any definite effect on the state of hypoxia of the brain tissue. After preliminary injection of cavinton, bemitil, and ethomersol, the fall of pO_2 in the cerebral cortex by the 30th minute of ischemia was significantly less (by 7, 11, and 40% respectively).

The antihypoxic action of the preparations may have been due to their ability to increase the blood supply to the brain, and to reduce the oxygen consumption of the tissue, and it may also have been realized through the facilitation of oxygen release by hemoglobin. Under the conditions studied, the main mechanism of the alleviation of hypoxia of the brain tissue is the ability of the substances to reduce affinity of Hb for O_2 and to increase the amount of oxygen given up to the brain tissue. This is confirmed by the close correlation found between the reduction of affinity of Hb for O_2 and the ability to limit the fall Of pO_2 in the cerebral cortex of the rats during acute ischemia, by the series of antihypoxic agents which we studied ($r = 0.87$; $p < 0.05$).

For a substance to exhibit antihypoxic activity in acute cerebral ischemia on account of a reduction of the affinity of Hb for O_2, this property must be sufficiently well exhibited. For instance, in the series of drugs tested, a protective effect was exhibited starting with cavinton, which lowered the HbO_2 level in the blood after saturation with the gas mixture used by 4.5%, or which increased p_{50} by 2.2 mm Hg. Meanwhile ascorbate, which lowered the HbO_2 level by 3.1%, with an increase of p_{50} by 1.1 mm Hg, exhibited only a tendency toward the alleviation of hypoxia.

Cavinton, sodium hydroxybutyrate, and bemitil, used in the present experiments, possessed properties of selective cerebral vasodilators, for they can increase the blood supply to the brain against a background of changes in the parameters of the systemic circulation below the level of significance or, at most, only moderate in degree [1, 8, 12]. However, in acute cerebral ischemia this property probably is not realized [8]. In rats after receiving an injection of bemitil, for instance, compared with the group of control animals no significant increase in cerebral blood flow could be found. The results were similar after the use of cavinton. Evidently in the early periods after cerebral ischemia, produced in rats by occlusion of both carotid arteries, a degree of hypoxia of brain tissue is created which leads to disturbance of the energy metabolism of the brain tissue and to acidosis, accompanied by vasomotor paralysis [9]. Under these conditions further dilatation of the cerebral vessels, even by the use of drugs with the properties of selective cerebral vasodilators is impossible.

Thus in acute cerebral ischemia, the preparations tested are able to alleviate hypoxia of brain tissue by their ability to reduce affinity of Hb for O_2 A contribution to the mechanism of the antihypoxic action of the compounds on the oxygen consumption of the brain requires further study.

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